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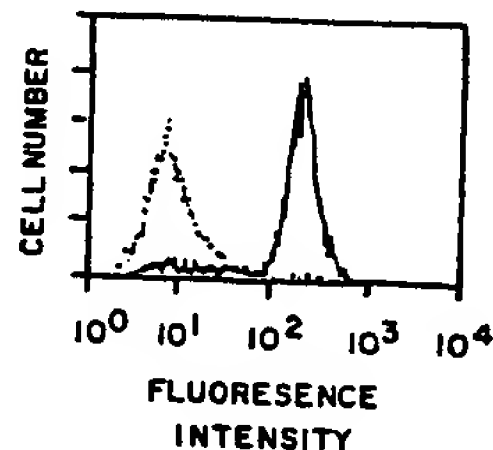
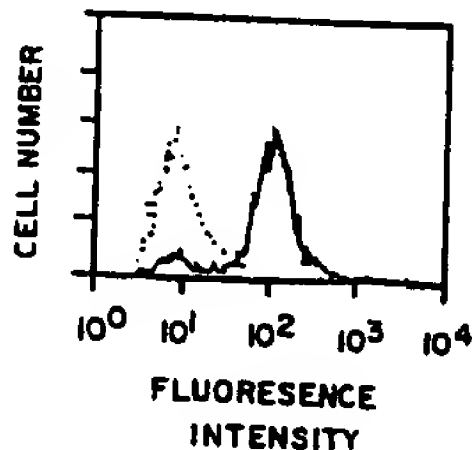
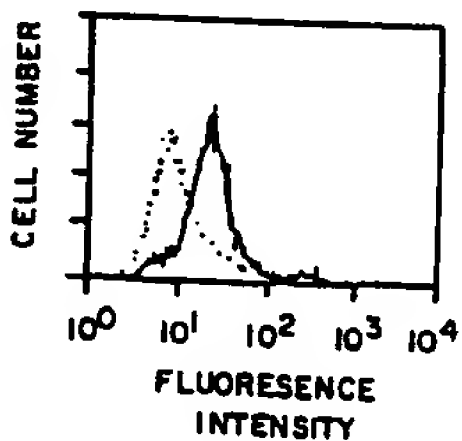
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(57) Abstract

Antibodies capable of binding to the  $\alpha$  subunit of the human GM-CSF receptor are described. These antibodies can inhibit the proliferation of cells whose growth is dependent upon the presence of human GM-CSF. Methods of assaying for the human GM-CSF receptor are also disclosed.

the antibody. Such peptides [Chiba, S., et al. Cell Reg. 1:327-335 (1990); Onetto-Pothier, N., et al., Blood 75:59-66 (1990)] can be synthesized by conventional means. For example, one can clone the  $\alpha$  receptor or screen for cells expressing the  $\alpha$  receptor. Preferably, one transforms a cell line with a vector encoding the  $\alpha$  subunit receptor. The transformed cells are then selected by standard means. The cell line is preferably from the same host strain being immunized to generate the antibody. Typically the vector used also contains a selectable marker so one can readily determine that the cell has been transformed. Alternatively, one can use labelled human GM-CSF to screen for cells expressing the receptor. The cells expressing the  $\alpha$  receptor are then preferably rescreened to obtain those cells expressing high levels of receptor. For example, one can subject the cells to fluorescence activated cell sorting (FACS) with a labelled GM-CSF, for example phycoerythrin-labelled hGM-CSF. Cells expressing high levels of the  $\alpha$  receptor are collected, incubated and resorted several times, e.g. 3 times, to obtain cells expressing very high levels of receptor.

The antibodies can be prepared by techniques well known to the skilled artisan. For example, cells having the receptor protein, the protein or an antigenic portion thereof can be conjugated to keyhole limpet hemocyanin (KLH) and used to raise an antibody in an animal such as a rabbit. Preferably, one uses the whole cell with a high level of receptors on it. Typically the peptide-KLH conjugate is injected several times over a period of about two months to generate antibodies. Antibodies are collected from serum by standard techniques and screened to find an antibody specific for the external portion of the receptor. Monoclonal antibodies can be produced in cells which produce antibodies and used to generate monoclonal antibodies by using standard fusion techniques for forming hybridoma cells [Kohler, G., et al. Nature 256:495 (1975)]. Typically this involves fusing an antibody producing cell with an immortal cell line such as a myeloma cell to produce the hybrid cell. Alternatively, monoclonal antibodies can be

produced from cells by the method of Huse, et al., Science 246:1275 (1989), both Kohler and Huse are incorporated herein by reference.

For example, hybridomas can be generated by immunization of mice with viable cells expressing the GM-CSF receptor. Preferably, these cells express the full length protein, although partial domains can also be used. Using the full length protein as an immunogen, it is possible to generate a collection of monoclonal antibodies with specificities that span the entire length of the protein. This is as opposed to the use of peptide immunogens or short polypeptides generated by prokaryotic systems, which present a more limited number of epitopes from the original protein and hence raise an immune response of more limited specificity. Furthermore, the protein should not be fully denatured, and should possess the native post translational modifications such as glycosylation.

The mice, for example, DBA/2 mice or SJL mice, can be immunized intraperitoneally (I.P.) with a sufficient number of viable cells of the host cell, which expresses high levels of the  $\alpha$  subunit receptor. The injection scheme used can be determined based upon the host. For example, i.p. injection without adjuvant into the DBA/2 mice every 4-6 weeks for a series of injections. Preferably, one would use 3-6 injections. One can check anti- $\alpha$  titer on cells expressing the  $\alpha$  receptor by FACS to see if the level of antibody produced is sufficient. With the injected SJL mice a cyclophosphamide injection intraperitoneally can be done one and two days following the primary injection. About two weeks following immunization, mice are then injected with a sufficient amount of cells expressing high levels of GM-CSF receptor and then allowed another two weeks at which time the entire procedure is repeated. Alternatively, with for example SJL mice, there can be 12 I.P. injections of different types of cells, which, however, express the receptor, every 1-2 weeks. This would be followed with a single large injection of receptor expressing cell. Intravenous injections can also be used if the cells expressing the receptor are of hematopoietic origin. Four days following the

last injection of the transformed cells, the animals are sacrificed and their spleens obtained for the first fusion.

Hybridomas are produced by fusing cells by standard techniques, such as from immunized mice with SP2/O myeloma cells by a polyethylene glycol (PEG) method. Cells are aseptically removed from immunized mice and a single cell suspension of the spleen cells obtained by perfusing the spleen with serum-free media (e.g., DMEM). Spleen cells and myeloma cells are mixed together at a ratio, for example, of 5 to 1, spleen cells to myeloma cells. The cells are then centrifuged and the supernatant removed by aspiration. The cells are then grown in medium by standard techniques. Hybridomas, which grow after the fusion procedure, are then screened for secretion of antibodies which show high levels of binding to cells having GM-CSF receptors and not receptor-free parental cells. Screening can be done on fixed cells or cell lysates or by cell surface immunofluorescence staining of live cells. Hybridomas that produce positive results are expanded and cloned by limiting dilution to assure that the cells and resulting antibodies are indeed monoclonal. Hybridoma colonies that test positive for these characteristics and presumably the presence of antibody to GM-CSF receptor are diluted in media to a concentration of, for example, 0.5 hybridoma cells per milliliter. Once colonies grow, the supernatants are again tested for the presence of antibody to the receptor. If the results are positive when tested by an ELISA assay, the colonies are cloned again by limiting dilution.

Preferred monoclonal antibodies include 1.013A, 207E1, 4A4C1, 11E7A1, and 12E7A1 which are mouse monoclonal antibody directed to the external portion of the receptor. Hybridomas expressing these monoclonal antibodies have been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and given Accession Nos. HB11198, HB11194, HB11195, HB11197, and

HB11196, respectively.

We have prepared at least three different types of antibodies. These antibodies are specific for the alpha chain of the human GM-CSF receptor. These antibodies provide three different types of interaction as measured by FACS. Fig. 1 shows FDCP1 [Dexter, T.M., et al., *J. Exp. Med.* 152:1036-1047 (1980)] cells express high levels of the recombinant GM-CSF $\alpha$  receptor (solid line) and wild type FDCP1 cells not expressing receptor (dotted line) incubated with tissue culture supernatant from hybridoma cells which produce anti GM-CSF antibodies for one hour at 4°. Three different levels of binding are observed. In Fig. 1A a low level of binding; while Fig. 1B shows a medium level, and Fig. 1C shows a high level of binding. All the cells were washed and incubated with label, for example, fluorescein label goat antimouse Ig Fab<sub>2</sub> fragment before being subjected to FACS analysis.

Such results suggest that there are three classes of antibody affinity, three different receptor epitopes or different forms of the receptor such as glycosylation differences or conformational changes induced by other subunits or proteins. Although not wishing to be bound by theory, we believe that it is the result of three different antibody specificities.

These antibodies are also able to inhibit the proliferation of cells that are dependent on the presence of hGM-CSF, and hence its effect on a cell. For example, the growth of human GM-CSF dependent cell lines such as TF-1 and MO7 and murine factor dependent cell lines, such as FDCP1 and Ba/F3 that express a functional human GM-CSF receptor is inhibited when grown in the presence of human GM-CSF. Interleukin-3 responsive growth is not affected by the antibody. This inhibition of effect can be shown using crude or purified antibodies individually or a pool of the antibodies. Although single antibodies can be used, in

certain embodiments one preferably uses a cocktail (pool) of such antibodies that bind to different epitopes to obtain the inhibition of proliferation effect. For example, the binding of a GM-CSF, such as a labelled GM-CSF such as  $^{125}\text{I}$ -GM-CSF, is blocked by such a pool. See, Figure 2 which measures a cell line having high receptor expression such as FDCP1 cells expressing human GM-CSF receptor alpha subunits. When such cell line was incubated in the presence of specified concentration of the labelled GM-CSF with or without a 150 fold excess of unlabelled GM-CSF, with or without pooled antibody, for example 30  $\mu\text{g/ml}$ , for an appropriate temperature for a sufficient time such as five hours at  $4^\circ\text{C}$ , the amount of antibody can be determined by standard techniques such as for example centrifuging of cells through 100% fetal calf serum with the pellet counted by use of a gamma counter. The results are set forth in the table below.

Table 1

$^{125}\text{I}$ -GM-CSF (M)	Specific Binding (cpm) <sup>a</sup>	Specific Binding <sup>b</sup> with Antibody (cpm)	% Inhibition <sup>c</sup>
$1.0 \times 10^{-9}$	1930	430	76
$5.0 \times 10^{-9}$	1342	407	70
$2.5 \times 10^{-10}$	318	82	75

<sup>a</sup> Specific binding - cpm bound minus cpm bound in the presence of unlabeled ligand.

<sup>b</sup> Specific binding with antibody - cpm bound in the presence of antibody minus cpm bound in the presence of antibody and unlabeled ligand.

<sup>c</sup> % decrease in  $^{125}\text{I}$ -GM-CSF binding caused by the antibodies.

In another embodiment the antibodies can be used to screen for cells expressing human GM-CSF receptor. Since the  $\alpha$  subunit is specific for the hGM-CSF receptor, as opposed to the  $\beta$  subunit, the antibodies to the  $\alpha$  subunit also determine the presence of the receptor. One can use any of the antibodies described herein. For example, the antibody and an iodinated avin. In one embodiment, one uses a cocktail of the different antibodies. Alternatively, one can

use one of the antibodies with a second labelled-antibody such as an anti-mouse antibody. In another embodiment, one can use a biotinylated antibody with a second label such as using biotinylated anti-GM-CSF receptor antibody. Such an antibody can be prepared by standard means, for example, by reaction of the antibody with NHS-LC-Biotin, and streptavidin allophycocyanin. By such a system one can test receptor expression by three color sorting. This permits a simple means to determine whether, for example, a stem cell fraction such as  $CD34^+CD38^-$  cells or a progenitor cell fraction such as  $CD34^+CD38^+$ , differ in expression of receptor or whether leukemia cells are lymphoid or myeloid. Other methods of testing cells to determine receptor expression can also be used. For example, autoradiography such as by iodinated antibody or iodinated streptavidin. Other assay methods using these antibodies are known such as a modification of that described by Koyama, H., et al., Anal. Biochem. 205:213-219 (1992).

These antibodies can also be used to deliver a target molecule to a desired cell expressing hGM-CSF receptors. For example one can conjugate the antibody to a cytotoxic molecule for example, a ricin molecule, to kill a target cell. Ricin can be linked to one of the described monoclonal antibodies by a number of means, such as using a non-reducible thioester bond using the methodology of Youle, E.J., Proc. Natl. Acad. Sci. USA 77:5483-5486 (1980). In another embodiment one can couple the protein to the desired molecule by a polylysine-conjugate adapting the methodology of Wu & Wu, J. Biol. Chem. 262:4429-4432, (1987). These conjugated antibodies can also be used to inhibit or kill the growth of myeloid leukemic cells which express the receptor. Thus this methodology should be useful in inhibiting or retarding leukemias as well as other diseases resulting from interaction of a molecule such as GM-CSF with the GM-CSF receptor.

The presence of the receptor and/or cells expressing the receptor can be determined by assaying for it using the antibodies as a probe.



In one preferred embodiment, one would use a quantitative immunoassay procedure. For example, one can determine whether the level of receptor on cells or in the body has increased or decreased when a treatment has begun. Thus, one can compare results against baseline levels obtained from the materials being sampled. Further, one can take samples from the same individual at various times to monitor continuing levels of expression.

For example, the expression of the CD34 molecule is a well established marker of hematopoietic differentiation. Thus, as you go from high CD34 expression (CD34<sup>+</sup>) to low or no CD34 expression (CD34<sup>-</sup>), you go from less differentiated cells, such as stem cells, to more differentiated cells, such as end-stage effector cells. We have found that within lineage-depleted (lin<sup>-</sup>) CD34<sup>+</sup> cells the presence or absence of GM-CSF $\alpha$  receptor (GMR $\alpha$ ) is indicative of the cells' differentiation state. For instance, lin<sup>-</sup>CD34<sup>+</sup> cells that do not have GMR $\alpha$ , when cultured with factors appropriate for the growth of immature cells, form many large colonies. This indicates that the cells undergo multiple cycles of division and establishes these cells as being less differentiated. In contrast, lin<sup>-</sup>CD34<sup>+</sup>GMR $\alpha$ <sup>+</sup> cells form few, very small colonies when cultured under the same conditions, thus indicating that the cells are more mature. We have obtained similar findings when the bone marrow cells were further fractionated according to the absence of CD38. The CD34<sup>+</sup>CD38<sup>-</sup> fraction of human bone marrow is highly enriched for the most primitive cells [Issaad, C., et al., Blood 81:2916-2924 (1991)]. Thus, CD34<sup>+</sup>CD38<sup>-</sup>GMR $\alpha$ <sup>+</sup> cells are capable of very little proliferation compared with CD34<sup>+</sup>CD38<sup>-</sup>GMR $\alpha$ <sup>-</sup> cells.

After bone marrow transplantation or chemotherapy, it is important to be able to determine the state of bone marrow recovery. At major transplant centers in the U.S., it has been reported that, depending on the type of transplant, between 5% and 20% of patients have poor engraftment. Similarly, the administration of intensive chemotherapy

often results in delayed bone marrow recovery, causing the patients to be at high risk for infection and preventing the administration of further life-saving chemotherapy. To assist bone marrow reconstitution and recovery, the patients can be given hematopoietic growth factors. By being able to identify individuals at an early stage that do not need this therapy, one can avoid needless therapy and also save money. Thus, by screening for the presence of GM-CSF  $\alpha$  subunit receptor in such individuals one can determine at an early stage whether the cells are regenerating.

For example, one can screen for the presence of receptor by using these antibodies to look at the cells, or by screening for the presence of such receptor in serum, either by looking at soluble receptor subunit or cleaved receptor subunit. Preferably, one looks for the presence of receptor in serum. More preferably, one looks for the presence of soluble receptor. One can compare the amount of receptor against a baseline level established to determine the presence of receptor on the marrow cells as they regenerate. The presence of receptor is indicative that the individual does not need therapy to enhance regeneration.

The soluble GM-CSF receptor may be detectable in body fluids such as blood, serum, plasma, urine, cerebrospinal fluid, supernatant from cell lysate breast aspirates and body tissues using these antibodies.

These antibodies can be used to determine the amount of receptor in a sample by contacting the sample, either body fluid or tissue, with at least one of the antibodies, preferably a monoclonal antibody, and determining whether binding has occurred. Preferably, one quantifies the amount of binding that occurs. As aforesaid, immunoreactive fragments of these antibodies can also be used and are included within the definition of antibody as used herein.

The GM-CSF receptor may be differentially expressed in normal and malignant cells. Tumors expressing the highest levels of receptor frequently are derived from cells which express high levels of cellular proteins and receptors. Thus, one can locate tumors by looking for high levels of binding of the present antibody. Then, by monitoring the level of receptor expression one can determine the response to therapy.

For example, we have shown that marrow cells in individuals having myeloid leukemia are positive for receptor, whereas those cells in individual's having lymphoid leukemia are typically negative for receptor. For instance, in 35 leukemia bone marrow samples randomly selected, all 21 of the samples of myeloid leukemia were positive for GM-CSF  $\alpha$  subunit receptor; whereas 12 of lymphoid leukemia marrow samples were negative, and the remaining two were questionably positive.

Further, GM-CSF interacts with cells via the GM-CSF receptors. High levels of GM-CSF have been shown to be involved in the establishment and progression of myeloid leukemias. Furthermore, its activity in synovial fluid in patients with rheumatoid arthritis suggest that the GM-CSF receptor is implicated in the etiology of this disorder. Various tumor cell lines of non-hematopoietic origin have been described as being responsive to GM-CSF. For example, small cell carcinoma of the lung, breast carcinoma cell lines, and SV40 transformed marrow stromal cell line, adenocarcinoma cell lines, tumors of neuroendocrine phenotype and neural crest origin, including malignant melanoma. Thus, by looking for the presence of receptor for GM-CSF on such cell lines, and comparing such levels of expression with a baseline one can determine differences. In addition, one can monitor progression of conditions by comparing the level of expression at different times. Human endothelial cells, trophoblast cells and placenta also express the receptor. The role of GM-CSF may also be important in the development of birth disorders. These antibodies provide a simple means for assaying for the effects of differing

and 5% human serum albumin. Liposomes may also be used as carriers. Additives, such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives, may also be used.

The preferred range of active ingredient in such vehicles is in concentrations of about 1 mg/ml to about 10 mg/ml. More preferably, about 3 mg/ml to about 10 mg/ml.

The present invention is further illustrated by the following examples. These examples are provided to aid in the understanding of the invention and are not to be construed as a limitation thereof.

Production of anti-GM-CSF receptor  $\alpha$  monoclonal antibody.

Expression of Cells containing A human GM-CSF Receptor

FDCP1 cells [Dexter, T.M., et al., J. Exp. Med. 152:1036-1047 (1980)] were electroporated with linear constructs of 60  $\mu$ gm of  $\alpha_2$  receptor subunit [Crosier K., et al., Proc. Natl. Acad. Sci. USA 88:7744-7748 (1991)] and 6  $\mu$ gm of the neomycin resistance gene both in the vector pmt21 at 980  $\mu$ F and 275 volts with the time constant set by the apparatus (Gene Pulser, Bio-Rad, Richmond, CA). The electroporated cells were first selected for their ability to grow in 1 mg/ml G418 (GIBCO, Grand Island, NY) and then in  $1 \times 10^{-8}$  M human GM-CSF. The cells were then subjected to fluorescence activated cell sorting (FACS, Becton-Dickinson, Rutherford, NJ) with phycoerythrin-labelled hGM-CSF (R and D Systems, Minneapolis, MN) according to the directions of the manufacturer, except that the number of cells used was doubled. Cells that expressed high levels of  $\alpha$  receptor (top 5-7%) were collected, expanded in the presence of hGM-CSF, and resorted 3 additional times to isolate cells with the highest receptor expression. Scatchard analysis using  $^{125}$ I-GM-CSF (New England Nuclear, Boston, MA) binding to these cells demonstrated

receptor expression in excess of 100,000 sites/cell.

Production of Antibodies to GM-CSF receptor  $\alpha$  subunit

The FDCP1 cells expressing high levels of  $\alpha$  subunit (FDC-GMR $\alpha$ ) were irradiated with 4,000 cGy and  $1-2 \times 10^5$  cells were injected intraperitoneally without adjuvant into DBA/2 mice every 4-6 weeks. After the fourth injection, the mice were checked for anti- $\alpha$  titers on FDC-GMR $\alpha$  by FACS using fluorescein isothiocyanate (FITC)-labelled goat anti-mouse Ig Fab<sub>2</sub> (Organon Technika, Durham, NC). An additional boost of FDC-GMR $\alpha$  was given both intravenously and intraperitoneally prior to the fusion.

Production of Monoclonal Antibodies

Spleens from 3 mice with the highest titers were fused to NS1 myeloma cells (American Type Culture Collection, Rockville, MD) by stirring in the presence of 50% PEG 1500 (Boehringer-Mannheim, Indianapolis, Ind.). The resulting hybridomas from each fusion were plated into twenty 96-well plates and were selected with HAT medium. All single-cell colonies were screened for the production of antibody that bound to FDC-GMR $\alpha$  but not to wild-type, non-transfected FDCP1 cells. All colonies meeting this criteria were then single-cell cloned two more times by limiting dilution. All subclones scored positive for anti-GMR $\alpha$  antibody production by FACS analysis. Antibodies expressed by five of these hybridoma were designated 1.013A1, 207E1, 4A4C1, 11E7A1, and 12E7A1. The 5 hybridomas expressing antibodies that were specific for human GM-CSF receptor  $\alpha$  subunit were deposited with the American Type Culture Collection (ATCC), 12301, Parklawn Drive, Rockville, MD 20852, pursuant to the Budapest Treaty and given Accession Numbers HB 11198, HB 11194, HB 11195, HB 11197, and HB 11196, respectively.

The FDCP1 cells ( $5 \times 10^5$ ) expressing high levels of the GM-CSF receptor and wild-type FDCP1 cells, ( $5 \times 10^5$ ) were incubated with tissue culture supernatant (0.1 ml) from hybridoma cells producing the antibodies for 1 hour at  $4^\circ\text{C}$ . The cells were washed and then incubated with fluorescein-labelled goat antimouse Ig F<sub>ab</sub><sub>2</sub> fragment and subjected to FACS analysis. Figs. 1A-C shows the different patterns of binding that were observed. In Fig. 1A, a low intensity binding was observed. Fig. 1B, a medium level of binding was observed and Fig. 1C, a high level of binding was observed.

#### Expression of GM-CSF $\alpha$ Subunit On Human Neutrophils

Normal peripheral blood was separated on a Ficoll gradient by centrifugation for 30 minutes at 600g. Distilled water was added to the pellet for 1 minute to lyse the red cells and then a 1/10 volume of 10x PBS was added to obtain isotonicity. A biotinylated GMR $\alpha$  antibody pool (containing equal amounts of all five antibodies) and biotinylated isotype control antibody were added to a final protein concentration of 20  $\mu\text{g/ml}$ . The cells were washed and then analysed on FACScan (Bectin-Dickerson). See Figure 5. The shift in the peak indicates that the GMR antibodies can detect the low level of receptor present on mature neutrophils. In Figure 5:

.....isotype control; \_\_\_\_\_ GM-CSF  $\alpha$  subunit receptor antibody.

#### Effect of Antibody On Cell Proliferation

The FDCP1 transfected cells (3000 cells/well) were cultured in the presence of either human GM-CSF (square in Fig. 2) or 10<sup>6</sup> WEHI condition media, which is a source of murine, IL-3 (diamond in Fig. 2) at the indicated concentrations for a pool of 5 purified antibodies for

36 hours at 37°C degrees. The cells were then labelled for 4 hours with <sup>3</sup>H-thymidine and the incorporated thymidine was measured. The results are shown in Fig. 2.

The above-described procedure was repeated using the 5 antibodies individually, either purified or not, i.e. the crude antibodies. These results show that all the individual antibodies were inhibitory.

In addition, the above-described procedure was repeated with the individual antibodies, either crude or purified, with the human GM-CSF dependent cell line TF-1 and MO7 as well as the murine factor dependent cell line BA-F3 which expresses functional transfected hGM-CSF receptors. The growth of these cell lines was also inhibited in the presence of GM-CSF, but not IL-3.

The antibody pool was able to inhibit the growth of human progenitor cells. Human bone marrow was obtained from healthy donors after informed consent. The mononuclear cells were isolated by centrifugation through Ficoll-Paque (Pharmacia) at 400 g for 40 minutes at room temperature. The cells were next incubated in Iscove's modified Dulbecco's medium (IMDM) supplemented with 15% fetal calf serum (FCS) for 2 hours at 37°C in tissue culture disks and the non-adherent cells were collected. The mature hematopoietic cells were removed by incubating the cells in monoclonal antibodies against CD2, CD5, CD11b, CD15, and CD19 as well as the My8 & 10F7 antibodies for 30 minutes at 4°C. The labelled lineage-positive cells were separated with a magnet and the remaining lineage-negative progenitor cells were saved. The progenitor cells were plated  $\pm$  20  $\mu$ g/ml of pooled antibody in serum-free methylcellulose cultures at  $10^3$  cells/well. These cultures contained stem cell factor (50 ng/ml) and GM-CSF (12 pM) or IL-3 (12 pM). The results are the mean number of granulocyte and

macrophage colonies from two dishes assayed after three weeks in culture.

	<u>GM-CSF</u>		<u>IL-3</u>	
Antibody	+	-	+	-
Colonies	5.0	14.0	20	16

The above procedure was repeated except the pooled cultures contained varying concentration of GM-CSF as indicated in Figure 4.

The experiment performed above was also done in the presence of varying concentrations of GM-CSF. The results are shown in Figure 4.

The inhibition of proliferation on these cells by the antibody appears to result, at least in part from blocking of ligand binding. The binding of GM-CSF to cells has been demonstrated to be inhibited by a pool of the purified antibodies. Alternatively, some of the antibodies may act at a site separate from ligand binding to cause the inhibitory effects.

#### Immunoprecipitation of Receptor

COS cells were transiently transfected with GM-CSF receptor  $\alpha$  subunit using the DEAE dextran technique. 48 hours later, they were incubated in the presence of 400 $\mu$ C/ml <sup>35</sup>S-methionine for 8 hours at 37°C degrees. The cells were then solubilized in 1% Triton, 30mM Tris pH 8.0, 150mM NaCl and protease inhibitors. A purified pool of the 5 antibodies was added to a final concentration of 30 $\mu$ g/ml for 2 hours at 4°C degrees and the complex was precipitated with sepharose-linked antimouse Ig immunoglobulin. The resulting material



was eluted with SDS sample buffer and labelled on a 7-13% acrylamide gradient gel and subjected to autoradiography. See Fig. 3. Lane 1 shows immunoprecipitation of cells transfected with the  $\alpha$  subunit receptor. Lane 2 shows immunoprecipitation of a mock-transfected cell. The bold arrow indicates a band in lane 1, not present in lane 2, which has the expected molecular weight of the  $\alpha$  subunit receptor. The low molecular weight region (50-70kD) of lane 1, which has an increased intensity over that observed in lane 2, probably represents partially processed receptor.

#### Inhibition of $^{125}$ I-GM-CSF Binding

The FDCP1 cells expressing the human GM-CSF  $\alpha$  subunit receptor were incubated in the presence of the indicated concentration of  $^{125}$ I-GM-CSF with or without 150-fold excess of unlabelled GM-CSF and with or without 30  $\mu$ g/ml of pooled antibody for 5 hours at 4°. See, Table 1, *supra*. The cells were then centrifuged through 100% fetal calf serum and the pellet counted on a gamma counter. The results are set forth in Table 1.

#### GM-CSF Receptor Expression On Lineage-Depleted Bone Marrow Cells

Normal human bone marrow was separated with Ficoll. The mononuclear cells at the interface were collected and then allowed to attach by incubating at 37°C in 10% fetal calf serum (FCS) and IMDM media. The non-adherent cells were then lineage-depleted of mature cells using the methodology of Bussolino et al., Nature 337:471-473, (1989) by incubating with murine monoclonal antibodies against CD2, CD5, CD11b, CD15, CD19, MY9 and glycophorin. The labeled cells were then removed by using magnetic beads conjugated with rabbit anti-mouse immunoglobulin antibody. The remaining cells were labeled with biotin-conjugated anti-GM-CSF  $\alpha$  subunit receptor antibody pool and FITC-labeled anti-CD34 antibody or isotype control antibodies, washed, incubated with phycoerythrin labelled streptavidin and then were

subjected to FACS analysis (Becton-Dickinson). See Figure 6.

In Figure 6, the X-axis indicates CD34 positivity, and the Y-axis indicates GM-CSF receptor positivity. Thus, in this experiment:

5.3% of the total immature cell population is CSF receptor positive, CD34-positive.

16.4% is CD34-positive, GM-CSF receptor-negative.

65.8% is CD34-negative, GM-CSF receptor-negative.

12.4% is CD34-negative, GM-CSF receptor-positive.

These results show that a portion of immature hematopoietic cells is GMR-positive.

The cells from the CD34-positive, GM-CSF receptor negative and CD34-positive GM-CSF receptor positive fractions from the cell sorter noted above were then cultured in methylcellulose containing IMDM, 10% FCS, and 10% FCS supplemented with Steel factor (kit ligand), IL-3 and GM-CSF, each at a concentration of  $5 \times 10^{-11}$  molar and erythropoietin at a concentration of 1 unit/ml. After incubating the cells for 16 days at 37°C, the cultures were scored for colony formation. In the dishes containing the cells from the CD34-positive, GM-CSF receptor negative fraction, there were  $388 \pm 59$  colonies formed per 1,000 cells plated. Colonies of all lineages were present, and many of these colonies were very large and of mixed cell type. In contrast, the cells from the CD34-positive, GM-CSF receptor positive fractions formed only  $34.6 \pm 3.5$  colonies per 1,000 cells plated. Most of the colonies formed were small myeloid colonies. There were no mixed-lineage colonies observed. This indicates that these cells were more

differentiated than the GM-CSF receptor negative cells. This data shows that the early progenitor cells which are involved in hematopoietic reconstitution express little if any GM-CSF receptor.

#### Screening of Malignant Cells

Leukemia cells were also tested for the expression of the GM-CSF receptor. 35 leukemia bone marrow samples were randomly selected from the bone marrow depository at the Dana Farber Cancer Institute immunophenotyping laboratory. The cells were first labeled with the anti-GM-CSF receptor antibody pool or isotype-control antibody, washed, and secondarily labeled with fluorescent-labeled anti-mouse immunoglobulin and subjected to FACS analysis. All 21 of the samples of myeloid leukemias were positive for GM-CSF receptor expression; 12 of 14 lymphoid leukemia marrow samples were negative, and the remaining two were questionably positive. These data together with the data from the cell sorting and cultures of normal bone marrow show that the antibodies to the  $\alpha$  subunit receptor should be able to screen for, target and/or selectively treat certain myeloid leukemias without significant toxicity to the immature progenitor cell populations.

It is evident that those skilled in the art given the benefit of the foregoing disclosure may make numerous modifications thereof and departures from the specific embodiments described herein without departing from the inventive concepts and the present invention is to be limited solely by the scope and spirit of the appended claims.

I Claim:

1. An isolated antibody which will specifically bind to an  $\alpha$  subunit of a human granulocyte macrophage colony stimulating factor (GM-CSF) receptor.
2. The antibody of claim 1, which is a monoclonal antibody.
3. A monoclonal antibody having the characteristic of binding to the same epitope as one of the monoclonal antibodies selected from the group consisting of 1.013A1, 207E1, 4A4C1, 11E7A1, and 12E7A1.
4. The antibody of claim 1, wherein the  $\alpha$  subunit has a molecular weight of about 50 to about 85 kD.
5. The antibody of claim 4, wherein the  $\alpha$  subunit has a molecular weight of about 75 to about 85 kD.
6. The antibody of claim 5, which is capable of inhibiting the proliferation of cells whose growth is dependent on the presence of human GM-CSF.
7. An assay for detecting or quantifying the presence of a receptor to human GM-CSF in cells or biological fluids of a human, which comprises:
  - (a) reacting the cell or biological fluid with the isolated antibody of claim 1, and
  - (b) determining whether or not binding has occurred.
8. The assay of claim 7, where one quantifies the binding that occurs.

9. The assay of claim 8, wherein one is quantifying the presence of the GM-CSF receptor on the cell.
10. A pharmaceutically acceptable composition of the antibody of claim 1 adapted to inhibit the proliferation of cells whose growth is dependent upon human GM-CSF.
11. A hybridoma cell line producing the monoclonal antibody of claim 2.
12. A hybridoma cell line expressing antibodies binding to the same epitope as one of the monoclonal antibodies expressed by the hybridoma having ATCC accession numbers HB11194, HB11195, HB11196, HB11197 or HB11198.
13. The assay of claim 7, where the  $\alpha$  subunit has a molecular weight of about 75 to about 85 kD.
14. The assay of claim 7, wherein the antibody is a monoclonal antibody having the characteristic of binding to the same epitope as one of the monoclonal antibodies selected from the group consisting of 1.013A1, 207E1, 4A4C1, 111EUA1 and 12E7A1.
15. The pharmaceutically acceptable composition of claim 10, wherein the antibody is a monoclonal antibody having the characteristic of binding to the same epitope as one of the monoclonal antibodies selected from the group consisting of 1.013A1, 207E1, 4A4C1, 111EUA1 and 12E7A1.
16. The assay of claim 7, wherein one is screening biological fluid for the presence of a soluble receptor to GM-CSF.
17. The assay of claim 7, wherein the human is a human who underwent

chemotherapy and one is screening for bone marrow cells to determine if receptor to human GM-CSF is present on CD34 negative marrow cells, wherein the presence of receptor is indicative of marrow regeneration.